

Effects of Growth Hormone (GH) and Growth Hormone Receptor (*GHR*) Gene Polymorphisms on Milk Production Traits in Holstein and Holstein x Chinese Yellow Cattle Crosses

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Abstract: The 3 years of data from 464 purebred Holstein and Holstein cross (unimproved Holstein-Chinese Yellow cattle crosses, improved Holstein-Chinese Yellow cattle crosses) cattle were used to evaluate effects of polymorphisms in both GH and GHR on milk yield and quality. Traits evaluated included 305 days milk, milk fat, milk protein and lactose yield and 305 days milk fat, milk protein and milk lactose percentage. Three genotypes and two genotypes were found within the *GH* and *GHR* genes, respectively. Based on mixed model least squares analyses (SAS®, Raleigh, NC, USA) there was evidence of genotypic difference for GH in 305 days milk yield for improved Holstein-Chinese Yellow cattle and purebred Holstein ($p < 0.10$) but only a weak trend for genotypic differences across breed groups ($p < 0.15$). Genotypic differences for GH in milk fat, protein and lactose yield were not evident in unimproved Holstein-Chinese Yellow cattle or purebred Holstein cattle ($p > 0.10$) but there were trends for genotypic differences in milk fat, protein and lactose yield in improved Holstein-Chinese Yellow cattle ($p < 0.10$). There was little evidence of genotypic differences for GHR in any trait ($p > 0.20$). Results from this research suggest polymorphisms in GH can affect milk yield and quality but such effects may be dependent on percentage Holstein in the cow.

Key words: Cattle, Holstein cattle, Chinese Yellow, *GH* gene, *GHR* gene, polymorphism, milk production

INTRODUCTION

The dairy industry in China is growing rapidly and there is considerable interest in small dairy production in rural China. Most smallholder dairies in rural China have limited income and feed resources. Consequently, it is often impractical for these households to either purchase or manage purebred Holstein and many households enter dairy production by crossing Holstein with Chinese Yellow cattle to produce crossbred cows used in milk production (Rae, 2008; Kemp *et al.*, 2011). However, there is little published information about milk yield and quality in the Holstein x Chinese Yellow cattle crosses nor is there information of the effects of polymorphisms in the Growth Hormone (GH) or Growth Hormone Receptor (*GHR*) genes on milk yield and quality in these crosses.

Growth Hormone (GH) is a single-chain peptide protein hormone which is secreted from the anterior

pituitary eosinophilic cells. Growth hormone has extensive physiological function in regulation of growth and acts on all tissues and cell types. The bovine *GH* gene (*bGH*), located on chromosome BTA19 includes five exons and four introns are comprised in a genomic sequence spanning 2856 bp (GenBank accession number M57764.1). Studies show that growth hormone plays a key role in feed utilization and mammary development while regulating metabolism, other physiological processes and immune response (Burton *et al.*, 1994). Therefore, the *GH* gene is considered to be an ideal candidate gene related to milk production traits, growth traits and immune response.

The *GH* gene sequence in yak (*Bos grunniens*) is shown to have a high homology to *Bos taurus* (Zhong *et al.*, 2007). Bai *et al.* (2009) analyzed the *GH* gene sequence of Gannan yak and reports two SNPs, a substitution in intron 3 (g.1694T>C) and a substitution in

exon 5 (g.2154G>A) due to transition (Glycine to Serine). Hao (2008) reports *GH* gene polymorphisms in intron 3 (g.1435T>C, g.1658A>G), in intron 4 (g.1918C>A) and in exon 5 (g.2291A>C and g.2386T>C), respectively. Yao *et al.* (1996) reports an allelic variant in the *GH* gene (p.Leu152Pro).

Growth Hormone Receptor (GHR) has a molecular weight between 100-130 kDa in most animals and is a transmembrane glycoprotein. The bovine Growth Hormone Receptor (*bGHR*) gene is located on chromosome BTA20 and consists of ten exons and nine introns. Growth hormone cannot be directly transported through the cell membrane so it must be bound to the cell membrane of the GHR for transport. Because polymorphisms in the *GHR* gene will affect the function of the *GH* gene and thus affect livestock productivity, the evaluation of the impact of polymorphisms in the *GHR* gene has practical significance in cattle breeding. Many studies have reported results evaluating the effects of genetic polymorphism in the bovine growth hormone receptor gene on production performance. Several studies report SNPs in the *GHR* gene partial coding regions and introns of Holstein-Friesian cows (Blott *et al.*, 2003; Viitala *et al.*, 2006; Wang, 2008). Sherman *et al.* (2008) reports an allelic variant (g.300G>A) in intron 4 (GenBank Acc. No. AY643807.1); Hao (2008) reports two SNPs, a substitution (g.41A>G) in exon 10 in the *GHR* gene (GeneBank Acc. No. AM16114) causing a change in gene expression from histidine to arginine and a substitution (g.70C>T), resulting in change in gene expression from arginine to cysteine.

MATERIALS AND METHODS

Animal description and sample collection: Milk and blood samples were collected from 464 Holstein or Holstein-cross cows with different percent of Holstein breeding and bull selection for milk yield potential (group) in Gansu Province of China (Table 1).

Holstein-cross cows were classified as unimproved hybrids (Holstein x Chinese Yellow cattle crosses with percent Holstein ranging from 50% in generation 0-93.75% in generation 2 with no sire of cow selection on milk yield potential) and improved hybrids (Holstein x Chinese

Yellow cattle crosses with percent Holstein ranging from 87.5% in generation zero to 98.44% in generation 2 with bulls used in production of cows selected for milk yield potential).

Blood samples were collected from venous blood in sterile 10 mL tubes containing 1.5 mL of ACD anticoagulant. Samples were frozen at -20°C, transported in insulated containers to the analytical laboratory and stored at -70°C until DNA extraction. The DNA was isolated from the blood samples using a Phenol/Chloroform Extraction Method (Chomczynski and Sacchi, 1987).

Primer design and PCR amplification: Primer pairs (P1F-CTTTCTAGCAGTCCAGCCTTGAC and P1R-AGAGCAGACCGTAGTCTTGAGC) were designed according to reference sequence (GenBank Acc No. M57764) for Polymerase Chain Reaction (PCR) amplification within the *GH* gene intron 4 and exon 5 in dairy cattle. Primer pairs (P2F-ATACTTGGGCTAGCAGTGACATTAT and P2R-CAACAAAGATGTAATGTAGAGCGA) (GeneBank Acc. No. AM161140) was used for PCR within the *GHR* gene exon 8. The PCR solution used in the analyses included 36 µL ultra-pure water, 5 µL 10×Taq Buffer (with 15M-MgCl₂), 4 µL dNTPs (2.5 M), 2 µL primer (12.5 pmol µL⁻¹), 1 µL TaqDNA Polymerase (2.5U µL⁻¹) and 2 µL genomic DNA (100 ng µL⁻¹). Amplification conditions for the *GH* gene PCR were as follows: 8 min at 94°C, 30 sec at 94°C, 35 sec at 60.8°C, 45 sec at 72°C, 35 cycles then 10 min at 72°C; amplification conditions for the *GHR* gene PCR were as follows: 5 min at 94°C, 30 sec at 94°C, 35 sec at 62.5°C, 45 sec at 72°C, 35 cycles then 10 min at 72°C (2720 Thermal Cycler, ABI Corp., USA). The resulting products were loaded to 1% agarose gels within 1×TAE and run at 90 V for 20 min. Polyacrylamide gel electrophoresis was performed with 1×TBE and results were observed after staining with the silver stain system. Polymorphisms of the GH and GHR were identified by PCR-SSCP and different genotypes were detected after DNA sequencing. Three samples from each genotype were sequenced.

Milk data: Milk yield and component production (milk fat yield, milk protein yield and lactose yield) and quality (milk fat, milk protein and lactose percent) over 305 days of lactation were measured according to DHI (Hu *et al.*, 2006).

Statistical analyses: Analyses of the data were done using PROC MIXED of SAS® (Cary, NC, USA). Linear models for milk yield, component production and quality

Table 1: Sample size for each generation and group

Generation	Groups		
	Low-hybrids [†]	Improved-hybrids [‡]	Purebred [‡]
0	50-75% (62)	87.5-93.75% (65)	100% (45)
1	75-87.5% (55)	93.75-96.875% (86)	100% (40)
2	87.5- 93.75% (35)	96.875 -<100% (42)	100% (34)

[†]Bulls for low-hybrid group (<87.5% Holstein) were unselected for milk yield, [‡]Bulls for improved-hybrids (>87.5 and <100% Holstein) and purebred group were selected for milk yield

included the fixed effects of generation, year nested in generation (repeated), genotype (GH or GHR), genotype x generation, group, genotype x group, generation x group, dam breed of cow nested in generation and group, group x production year nested in generation, age of cow (linear), group x age of cow (linear), generation x age of cow (linear), year x age of cow (linear) nested in generation and dam breed of cow x age of dam (linear) nested in generation and group. Linear models were reduced as appropriate when the probability associated with the F-tests of subject effects was >0.25 . Linear contrasts among least square means were done using least significant differences at $p<0.10$ and $p<0.05$.

RESULTS

Polymorphisms: The PCR amplification products of the *GH* and *GHR* gene were tested using SSCP and results are shown in Fig. 1 and 2. A total of three genotypes (two homozygotes and one heterozygote) and two genotypes (one homozygote and one heterozygote) were found within the *GH* and *GHR* gene of 464 dairy cows, respectively, according to PCR-SSCP. Sequence diagrams for the *GH* and *GHR* are shown in Fig. 3 and 4.

Sequencing results show that an allelic variation was found (g.2017C>T) in the *GH* gene (GenBank Acc. No. M57764.1), resulted in three genotypes designated as CC, TT and TC, respectively.

The sequencing results of SSCP products for the *GHR* showed a substitution (g.4926A>T) in the *GHR* gene (GenBank Acc. No. AM161140), suggesting two genotypes of the *GHR* gene in these data, designated AT and AA, respectively. This was a sense mutation resulting in tyrosine rather than phenylalanine as the amino acid gene product.

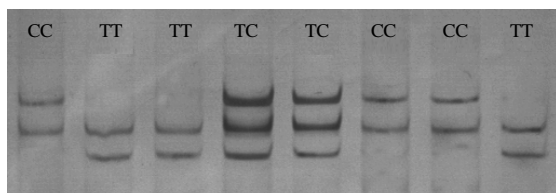


Fig. 1: *GH* gene amplification products in SSCP

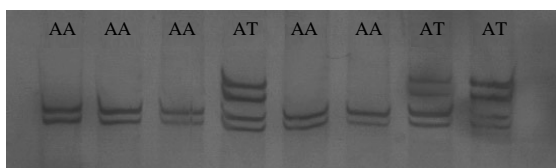


Fig. 2: *GHR* gene amplification products in SSCP

Genotypic and allelic frequencies are shown in Table 2. In the all of the populations, the growth hormone CC genotype and C allele had the greatest frequency while the growth hormone receptor AT genotype and A allele had the greatest frequency. Genetic diversity estimates (homozygosity, heterozygosity, effective number of alleles and polymorphism information content) within the 3 populations of dairy cows for the *GH* and *GHR* genes are given in Table 3. Heterozygosity estimates for *GH* were similar among the unimproved hybrid, improved hybrid and purebred groups. Heterozygosity estimates for *GHR* were similar for the unimproved hybrids and purebreds but appeared higher for the improved hybrids, giving a larger number of effective alleles for this group (3.17). Polymorphism information content was similar among groups for each gene and was moderate (Vaiman *et al.*, 1994).

Milk production traits: Probabilities associated with F-tests for tests of fixed effects in the linear models for milk yield and quality for the analyses of the effects of polymorphisms in the *GH* gene are given in Table 4. There

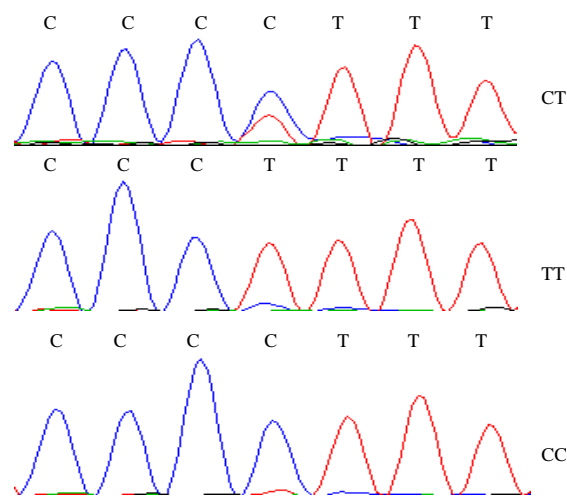


Fig. 3: Sequencing diagrams of *GH* gene

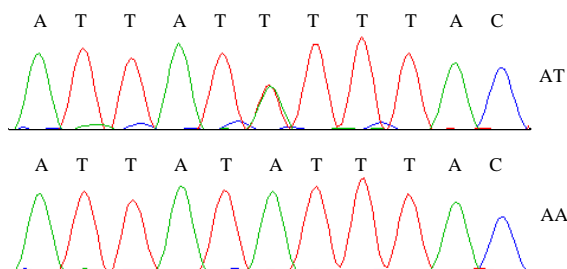


Fig. 4: Sequencing diagrams of *GHR* gene

Table 2: Genotypic and allelic frequencies and numbers of *GH* gene and *GHR* gene

Levels of breeding	Low-hybrids [†]	Improved-hybrids [‡]	Purebreds [‡]
GH-SNP site (Genotypes)			
CC	0.5472 (58)	0.4324 (96)	0.4559 (62)
TT	0.1132 (12)	0.2072 (46)	0.1765 (24)
CT	0.3396 (36)	0.3604 (80)	0.3676 (50)
Alleles			
C	0.7170	0.6126	0.6397
T	0.2830	0.3874	0.3603
GHR-SNP site (Genotypes)			
AA	0.4151 (44)	0.3153 (70)	0.3676 (50)
AT	0.5849 (62)	0.6847 (154)	0.6324 (86)
Alleles			
T	0.2925	0.3423	0.3162
A	0.7075	0.6577	0.6838

[†]Bulls for low-hybrid group (<87.5% holstein) were unselected for milk yield. [‡]Bulls for improved-hybrids (≥87.5 and <100% Holstein) and purebred group were selected for milk yield

Table 3: Population genetic indexes for the *GH* and *GHR* genes in dairy populations

Genes	Groups [†]	n	Ho	He	Ne	PIC
<i>GH</i>	Unimproved hybrid	152	0.66	0.34	1.51	0.32
	Improved hybrid	193	0.64	0.36	1.56	0.36
	Purebred	119	0.63	0.37	1.58	0.35
<i>GHR</i>	Unimproved hybrid	152	0.42	0.58	2.41	0.33
	Improved hybrid	193	0.32	0.68	3.17	0.35
	Purebred	119	0.37	0.63	2.72	0.34

[†]Bulls for low-hybrid group (<87.5% holstein) were unselected for milk yield. Bulls for improved-hybrids (≥87.5 and <100% holstein) and purebred group were selected for milk yield

Table 4: Probabilities associated with F-tests of fixed effects for milk production traits for *GH* gene analyses

Factors	305 days milk yield	305 days milk fat yield	305 days milk protein yield	305 days lactose yield	305 days milk fat percentage	305 days milk protein percentage	305 days lactose percentage
GEN	0.12	0.48	0.45	0.38	0.87	0.68	0.32
PRODYR (GEN)	0.00	0.00	0.00	0.00	0.39	0.22	0.06
GH-SNP	0.87	0.97	0.97	0.96	0.06	0.21	0.84
GEN×GH-SNP	0.67	0.50	0.48	0.44	0.06	0.06	0.04
GROUP	0.03	0.24	0.26	0.29	0.38	0.68	0.93
GROUP×GH-SNP	0.13	0.23	0.22	0.20	0.10	0.22	0.60
GROUP×GEN	0.05	0.04	0.04	0.04	0.62	0.73	0.75
BOD (GROUP×GEN)	0.22	0.09	0.10	0.11	0.97	0.95	0.77
PRODYR×GROUP (GEN)	0.31	0.63	0.61	0.56	0.97	0.92	0.29
AOD	0.00	0.00	0.00	0.00	0.10	0.19	0.45
AOD×GEN	0.18	0.34	0.35	0.39	0.79	0.99	0.86
AOD×GROUP	0.03	0.08	0.07	0.06	0.34	0.86	0.55
AOD×PRODYR (GEN)	0.08	0.25	0.24	0.22	0.43	0.41	0.19
AOD×BOD (GROUP×GEN)	0.03	0.04	0.04	0.05	0.92	0.93	0.75

GEN: Generation Effect including the generation zero, one and two; GH-SNP: Genotypes of the *GH* gene; GROUP: Group including low-hybrid, improved-hybrid and pure Holstein; BOD (GROUP×GEN): Dam breed of cow nested in generation and group; PRODYR (GEN): Year nested in generation; AOD: Age of cow

Table 5: Least square means and standard errors for milk production traits with different GH genotypes (kg or %)

Groups [†]	Genotype	305 days milk yield	305 days milk fat yield	305 days milk protein yield	305 days lactose yield	305 days milk fat percentage	305 days milk protein percentage	305 days lactose percentage
Low-hybrid	TT	5353±1233	250±51	253±52	261±54	3.81±0.04 ^{bn}	3.87±0.05 ^{bn}	4.00±0.08
	CC	5627±354	243±18	245±18	249±19	3.92±0.01 ^{am}	3.96±0.02 ^{am}	4.02±0.03
	TC	5667±455	247±22	250±23	254±23	3.94±0.02 ^{am}	3.98±0.02 ^{am}	4.05±0.03
Improved-hybrid	TT	6847±428 ^a	300±16 ^a	304±17 ^a	312±17 ^a	3.92±0.01	3.98±0.01	4.07±0.02 ^{ab}
	CC	6474±303 ^{ab}	291±11 ^{ab}	296±11 ^{ab}	304±11 ^a	3.93±0.01	3.98±0.01	4.08±0.01 ^a
	TC	6101±358 ^b	269±14 ^b	273±14 ^b	279±14 ^b	3.92±0.01	3.96±0.01	4.05±0.02 ^b
Purebred	TT	6021±551 ^{ab}	253±25	256±25	262±26	3.94±0.02	3.98±0.02	4.06±0.03
	CC	6181±286 ^b	260±12	263±12	269±13	3.94±0.01	3.99±0.01	4.07±0.02
	TC	6860±370 ^a	285±16	288±16	295±17	3.92±0.01	3.97±0.02	4.06±0.02

[†]Bulls for low-hybrid group (<87.5% holstein) were unselected for milk yield. Bulls for improved-hybrids (≥87.5 and <100% holstein) and purebred group were selected for milk yield. ^aGenotype means in the same group and column differ (p<0.10), ^{am}Genotype means in the same group and column differ (p<0.05)

were trends for dam breed differences within group and generation for 305 days milk fat yield, milk protein yield and lactose yield (p≤0.11). There was evidence that group effects were not consistent across generation for 305 days milk yield, milk fat yield, milk protein yield and lactose yield (p≤0.05). There was a weak trend for GH-SNP differences in 305 days milk yield and milk fat percentage to vary with group (p≤0.13) and a trend for GH-SNP differences in 305 days milk fat percentages to vary with generation (p≤0.06).

Least squares means and standard errors for milk, milk fat, milk protein, lactose yield, percent milk fat, protein and lactose for each GROUP and GH genotype are shown in Table 5. Genotypic differences for milk yield were not evident in the unimproved hybrid group (p>0.10), there was a trend for genotype TT to be greater than TC in the improved hybrid group (p<0.10) and a trend for genotype TC to be greater than CC in the purebred group (p<0.10). Genotypic differences for milk fat yield, protein yield and lactose yield were not evident in unimproved hybrid cattle or purebred cattle (p>0.10) but milk fat yield and protein yield differences in TT trended greater than TC and lactose yield differences in TC trended lesser than TT and CC in the improved hybrid cattle (p<0.10).

Table 6: Probabilities associated with F-tests of fixed effects for milk production traits for *GHR* gene analyses

Factors	305 days milk yield	305 days milk fat yield	305 days milk protein yield	305 days lactose yield	305 days milk fat percentage	305 days milk protein percentage	305 days lactose percentage
GEN	0.17	0.60	0.56	0.49	0.95	0.58	0.35
PRODYR (GEN)	0.00	0.00	0.00	0.00	0.23	0.21	0.10
GHR-SNP	0.20	0.23	0.23	0.24	0.25	0.52	0.93
GEN×GHR-SNP	0.45	0.46	0.46	0.46	0.06	0.08	0.27
GROUP	0.02	0.20	0.21	0.24	0.76	0.81	0.65
GROUP×GHR-SNP	0.81	0.94	0.94	0.93	0.81	0.79	0.88
GROUP×GEN	0.09	0.06	0.06	0.06	0.56	0.70	0.73
BOD(GROUP×GEN)	0.14	0.08	0.09	0.10	0.84	0.84	0.71
PRODYR×GROUP (GEN)	0.29	0.66	0.64	0.60	0.84	0.92	0.40
AOD	0.01	0.00	0.00	0.00	0.44	0.59	0.87
AOD×GEN	0.17	0.37	0.39	0.45	0.58	0.83	0.67
AOD×GROUP	0.03	0.07	0.06	0.05	0.89	0.57	0.28
AOD×PRODYR (GEN)	0.08	0.26	0.25	0.22	0.32	0.47	0.30
AOD×BOD (GROUP×GEN)	0.04	0.04	0.04	0.05	0.52	0.59	0.50

GEN: Generation effect including the generation zero, one and two; GH-SNP: Genotypes of the *GH* gene; GROUP: Group including low-hybrid, improved-hybrid and pure Holstein; BOD (GROUP×GEN): Dam breed of cow nested in generation and group; PRODYR (GEN): Year nested in generation; AOD: Age of cow

Table 7: Least square means and standard errors for milk production traits with different *GHR* genotypes (kg or %)

Groups [†]	Genotype	Milk yield	Milk fat yield	Milk protein yield	Lactose yield	305 days milk fat percentage	305 days milk protein percentage	305 days lactose percentage
Low-hybrid	AA	5361±393	232±21	235±22	239±23	3.94±0.02	3.98±0.02	4.04±0.03
	AT	5817±362	246±17	248±17	252±17	3.92±0.01	3.96±0.02	4.03±0.02
Improved-hybrid	AA	6205±350	278±12	282±12	289±12	3.93±0.01	3.98±0.01	4.07±0.02
	AT	6495±308	295±12	299±12	307±12	3.92±0.01	3.97±0.01	4.07±0.02
Purebred	AA	6304±322	262±15	265±16	271±16	3.94±0.01	3.98±0.02	4.05±0.02
	AT	6384±293	271±12	274±12	280±12	3.94±0.01	3.98±0.01	4.06±0.02

[†]Bulls for low-hybrid group (<87.5% Holstein) were unselected for milk yield. Bulls for improved-hybrids (≥87.5 and <100% holstein) and purebred group were selected for milk yield

There was a trend ($p<0.10$) for percent milk fat to be lesser in the TT genotype compared to CC and TC genotypes in the unimproved Hybrid group (Hy). There was also evidence for percent milk protein to be lesser in the TT genotype compared to CC ($p<0.10$) and TC ($p<0.05$) genotypes in the unimproved Hybrid group (Hy).

In Improved hybrids (Ihy) lactose percentage tended to be higher in CC than TC ($p<0.10$). There was little evidence ($p>0.10$) of any other genotypic differences with breed groups.

The probabilities associated with F-tests for tests of fixed effects in the linear models for milk yield and quality for the analyses of the effects of polymorphisms in the *GHR* gene are given in Table 6. There was little evidence of interactions of the GHR genotype with either group or generation nor was there strong evidence of significant main effects of GHR genotype ($p>0.20$). The exception was a trend for differences in GHR-SNP for 305 days milk fat percentage and milk protein percentage to vary with generation ($p\leq 0.08$). Least squares means and standard errors for milk, milk fat, milk protein, lactose yield, percent milk fat, protein and lactose for each GROUP and GHR genotype are given in Table 7. Numerically, the AT genotype for the GHR was greater than the AA genotype for milk yield milk fat yield protein yield and lactose yield. There were no indications of important genotypic differences in percent milk fat, protein or lactose for GHR within the breed groups.

DISCUSSION

Polymorphisms and gene frequencies: He (2005), using PCR-RFLP, report similar GH genotypes as were found in this study in the *GH* gene intron 4 and partial sequence of exon 5 on Chinese Holstein and detected two genotypes, AA and AB. Gao (2004) and Hao (2008) report polymorphisms at the 1978, 1947, 1918 bp of the *GH* gene intron 4 in Simmental cattle, Luxi cattle, Nanyang cattle and prairie Red bull indicating that variation in intron 4 of the GH exists in these different breeds. These results for the GH genotypic and allelic frequencies were similar with reports of Yao *et al.* (1996) and Zhang (2007).

Results for the GHR allelic frequencies were consistent with Wang (2008) with allelic frequencies of A greater than T but different from Viitala *et al.* (2006) in Finland Aryshire cattle where the frequency of A is less than T. In the study, the TT genotype of the *GHR* gene was not identified. Gao (2004) did not detect polymorphism at this loci in Chinese Yellow cattle and did not identify the homozygous TT genotype. However, Wang (2008) identifies the TT genotype at this site in Chinese Holstein.

Effects of polymorphisms on milk traits growth hormone: He (2005) reports yak growth hormone gene polymorphisms in exon 5 that affected 305 days milk yield. Lee *et al.* (1996) reports a reduction in milk yield

associated with a polymorphism in the *GH* gene and that another polymorphism is significantly related to milk fat content of Holstein. Falaki *et al.* (1996) found that GH allele MspI (-) is related to higher milk fat percentage, milk protein and milk production of Italian dairy cattle. Yao *et al.* (1996) studied the *GH* gene polymorphisms and finds the *GH* gene allele MspI (+) is associated with a favorable substitution effect of 300 kg for milk yield, 8 kg for fat content and 7 kg for protein per lactation. Lagziel *et al.* (1996), reports 5 haplotypes within the *bGH* gene of Israel Holstein dairy cows, one of which is significantly correlated to milk protein percentage.

The current study detected one polymorphic site in the *GH* gene in introns 4 and *GHR* gene in exon 8, respectively. The polymorphism in the *GH* gene was weakly associated with 305 days milk yield ($p < 0.15$) with genotype TT having a trend for a positive effect on milk production ($p < 0.10$) compared to TC in the improved hybrid breed group. He (2005) reports no significant differences in 305 days milk yield of Holstein among AluI RFLP restriction enzyme sites of different genotypes within the *GH* genes introns 4 and exon 5 partial sequences. Recent studies show that the intron in the genome has an important role in maintaining specific gene functions. The intron can regulate gene expression; many genes expression functions are associated with the intron (Balvay *et al.*, 1992; Hormuzdi *et al.*, 1998; Banos *et al.*, 2008). These results suggest that the *GH* gene is one of the major genes of the milk production traits of Holstein and Holstein x Chinese Yellow cattle dairy cows or is closely linked with one of the major genes and may be useful for milk traits as site useful in marker assisted selection. These results also indicate that further studies are needed at the DNA level to obtain more definite conclusions concerning the molecular events underlying the *GH* gene intron as well as its effects, direct or via a linked QTL, on milk production traits.

Growth hormone receptor: Much of the literature for the bovine *GHR* gene has focused on the relationships of gene polymorphisms to growth traits and few studies have reported effects of polymorphisms in *GHR* on milk production traits in dairy cattle. Banos *et al.* (2008) reports a sense mutation in the *GHR* gene at position 4962 in Holstein with a positive effect on milk yield, possibly because of the production of tyrosine rather than phenylalanine by the mutation. Falaki *et al.* (1996) reports that nine RFLP genotypes were found in the encoding C terminal sequence of the bovine *GHR* gene and polymorphisms at this site are related to percent milk

protein of Italian Holstein. The results of the study indicated that polymorphisms at site 4962 of the *GHR* gene had little effect on 305 days milk yield, 305 days milk fat, 305 days milk protein and 305 days lactose content ($p > 0.20$) in Holstein and Holstein x Chinese Yellow cattle.

These results are consistent with Wang (2008) for milk yield and protein who report no differences among genotypes at site 4962 in the *GHR* gene on milk yield and milk protein percentage of Holstein. However, Wang (2008) reports genotypic differences for percent milk fat at site 4962 of the *GHR* gene and Viitala *et al.* (2006) reports a polymorphism at site 4962 of the *GHR* gene that affected milk yield and composition of Holstein. There is a need for further research on the association site 4962 of the *GHR* gene with milk production traits in dairy cattle.

CONCLUSION

However, there has been little research published in determination of polymorphisms in the growth hormone or growth hormone receptor genes in crosses of Holstein and Chinese Yellow cattle and the effects of these polymorphisms on milk yield and quality. Consequently, polymorphisms in the *GH* and *GHR* genes of dairy cows with different genetic potentials and breed composition were studied using the PCR-SSCP Method by evaluating the effects of these polymorphisms on performance in Holstein and Holstein x Chinese Yellow cattle crosses.

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REFERENCES

- Bai, J.J., J.Q. Wang, J. Hu and Y.Z. Luo, 2009. SNPs of the growth hormone gene in Gannan yak. *China Cattle Sci.*, 35: 1-5 (In Chinese).
- Balvay, L., D. Libri, M. Gallegol and M.Y. Fiszman, 1992. Intronic sequence with both negative and positive effects on the regulation of alternative transcripts of the chicken β trophmyosin transcripts. *Nucl. Acids Res.*, 20: 3987-3992.
- Banos, G., J.A. Woolliams, B.W. Woodward, A.B. Forbes and M.P. Coffey, 2008. Impact of single nucleotide polymorphisms in leptin, leptin receptor, growth hormone receptor and diacylglycerol acyltransferase (DGAT1) gene loci on milk production, feed and body energy traits of UK dairy cows. *J. Dairy Sci.*, 91: 3190-3200.

- Blott, S., J.J. Kim, S. Moiso, A.S. Kuntzel and A. Cornet, 2003. Molecular dissection of a quantitative trait locus a phenylalanine to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *J. Gen.*, 163: 253-266.
- Burton, J.L., B.W. McBride, E. Block, D.R. Glimm and J.J. Kennelly, 1994. A review of bovine growth hormone. *Can. J. Anim. Sci.*, 74: 167-201.
- Chomczynski, P. and N. Sacchi, 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, 162: 156-159.
- Falaki, M., N. Gengler, M. Sneyers, A. Prandi, S. Massart, A. Formigoni and A. Burny, 1996. Relationships of polymorphisms for growth hormone and growth hormone receptor genes with milk production traits for Italian Holstein-Friesian bulls. *J. Dairy Sci.*, 79: 1446-1453.
- Gao, X., 2004. Molecular markers of candidate genes of growth development traits in Cattle. Master's Thesis, Northwest Sci-Tech A and F University, China (In Chinese).
- Hao, L.L., 2008. Association of the *GH* and *GHR* gene polymorphisms with carcass traits in pasture red cattle. Master's Thesis, Jilin University, China (In Chinese).
- He, H., 2005. Association of polymorphisms of three genes with production traits in Chinese Holstein and Yak. Master's Thesis, Sichuan University, China (In Chinese).
- Hormuzdi, S.G., R. Penttinen, R. Jaenisch and P. Bornstein, 1998. A gene-Targeting approach identifies a function for the first intron in expression of the $\alpha 1(I)$ collagen gene. *J. Mol. Cell. Biol.*, 18: 3368-3375.
- Hu, H.Y., G.H. Liu, D.L. Zhang and T. Wang, 2006. The establishment and application of DHI system. *J. Shandong J. Anim. Husbandry Vet. Sci.*, 6: 4-7.
- Kemp, D.R., D.L. Michalk, W.J. Bai, C. Brown and H.M. Dong *et al.*, 2011. Development of sustainable livestock systems on grasslands in North-Western China. ACIAR Proceedings No. 134. Australian Centre for International Agricultural Research, Canberra, pp: 12-177.
- Lagziel, A., E. Lipkin and M. Soller, 1996. Association between SSCP haplotypes at the bovine growth hormone gene and milk protein percentage. *J. Genet.*, 142: 945-951.
- Lee, B.K., G.F. Lin, B.A. Crooker, M.P. Murtaugh and L.B. Hansen *et al.*, 1996. Association of somatotropin (BST) gene polymorphism at the 5th exon with selection for milk yield in Holstein cows. *J. Domestic Anim. End.*, 13: 373-381.
- Rae, A., 2008. Chinas agriculture, smallholders and trade: Driven by the livestock revolution. *Agric. Resour. Econ.*, 52: 283-302.
- Sherman, E.L., J.D. Nkrumah, B.M. Murdoch, C. Li, Z. Wang, A. Fu and S.S. Moore, 2008. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2 and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency and carcass merit in beef cattle. *J. Anim. Sci.*, 86: 1-16.
- Vaiman, D., D. Mecier, G.K. Moazami, A. Eggen and R. Ciampolini, 1994. A set of 99 cattle microsatellites: Characterization, syntenic mapping and polymorphism. *Mamm. Genome.*, 5: 288-297.
- Viitala, S., J. Szyda, S. Blott, N. Schulman and M. Lidauer, 2006. The role of the bovine growth hormone receptor and prolactin receptor genes in milk, fat and protein production in finnish ayrshire dairy cattle. *J. Genet* 173: 2151-2164.
- Wang, L.J., 2008. Association of prolactin gene and *GHR* gene polymorphisms with milk yield traits. Master's Thesis, ShanDong University, China (In Chinese).
- Yao, J.B., S.E. Aggreg, D. Zadworny, J.F. Hayes and U. Kuhnlein, 1996. Sequence variation in the bovine growth hormone gene characterized by SSCP analysis and their association with milk production traits in Holsteins. *J. Genetics.*, 144: 1809-1816.
- Zhang, H.R., 2007. Relationship of growth gene SNP with growth development, milk yield and birth weight of Holstein. Master's Thesis, Gansu Agriculture University, China, (In Chinese).
- Zhong, J.C., Z.J. Ma, Z.X. Ou and S.G. Liu 2007. Cloning and sequencing of GH, H-FABP and HSP72 genes in yak *Can. J. Anim. Sci* 87: 503-510.